Clinical results of microfluidic antibody-independent peripheral blood circulating tumor cell capture for the diagnosis of lung cancer

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Objectives: The ability to capture and characterize peripheral blood circulating tumor cells has the potential for the development of a blood test for cancer. The aim of this study was to evaluate the diagnostic performance of microfluidic technology as a proof-of-concept study.

Methods: Blood from patients undergoing surgery for known or suspected lung cancer was obtained and processed using a microfluidic biochip. Diagnostic performance was evaluated against the reference of cancer identified within surgically obtained formalin-fixed paraffin embedded specimens reported by a principal pathologist. Agreement was assessed in a sample reported by a second independent pathologist. Sensitivity- and specificity-weighted analyses were undertaken.

Results: From March 2011 to October 2012, 46 patients at our institution donated blood for research. Cancer was the underlying diagnosis in 43 (94%); 34 (79%) of the patients had primary lung cancer. The proportion of patients with cancer in which atypical cells suspicious for cancer were identified on hematoxylin and eosin staining was 16/43 (37%) by the principal pathologist and 10/17 (59%) by the second pathologist. On sensitivity-weighted analysis, the sensitivity of the biochip was 54% (95% confidence interval [CI], 37-72) and the specificity was 33% (95% CI, 2-91). On specificity-weighted analysis, the sensitivity was 43% (95% CI, 21-71) and the specificity was 100% (95% CI, 5-100).

Conclusions: This work highlights the potential of microfluidic technology to develop a blood test for the diagnosis of cancer using peripheral blood; conventional clinical criteria can be used as a proof-of-concept of what may be possible with today's technology. (J Thorac Cardiovasc Surg 2014;147:1936-8)

The ability to capture and characterize circulating tumor cells (CTCs) has the potential to lead to clinical applications in cancer that include disease monitoring, refining prognosis, and predicting treatment response.

Several different technologies are being developed to capture peripheral blood CTCs either by size filtration, antibody recognition (followed by immunomagnetic separation), or direct blood smear analysis.[1] Indirect identification of CTCs in peripheral blood by real-time polymerase chain reaction is also possible.[2] Recent advances in microfluidic engineering have facilitated the development of an antibody-independent, microfluidic, biochip designed to capture peripheral blood CTCs.[3] Initial tests developed using spiked in cancer cells (from cell lines) were undertaken with good results, however the technology has yet to be evaluated in a clinical setting.

In this study, the microfluidic biochip was evaluated to determine if it is possible to capture and identify cancer cells circulating in the blood of patients using conventional cytomorphologic criteria with hematoxylin and eosin (H&E) staining as a proof-of-concept study in a series of patients undergoing surgery for lung cancer.

METHODS

The study was undertaken at The Royal Brompton and Harefield NHS Foundation Trust. The project was approved under the auspices of the National Institute of Health Biomedical Research Unit Advanced Disease Biobank (NRES 10/H0504/9).

From March 2011 to February 2012, blood samples were obtained from 46 patients and processed using the ClearCell CTChip (Clearbridge BioMedics, Singapore) by filtering 1 mL of blood within a microfluidic biochip and trapping cells within the 900 chambers; the samples were subsequently stained using H&E and reported in-chip[3] as previously described.[4] Patient blood samples were acquired before surgery (by venesection or central venous catheter) and samples were processed on the day of receipt.

After H&E staining, the biochips were assessed by a principal pathologist (A.G.N). When present, enlarged nucleated cells, either single or in clusters, were classified as atypical cells suspicious for cancer.

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Abbreviations and Acronyms
CTC = circulating tumor cell
H&E = hematoxylin and eosin
SD = standard deviation

To assess interobserver agreement, 18 biochips were also reviewed in a blinded fashion independently by a second pathologist. Agreement was reported by means of a kappa statistic and degree of agreement was classified according to the criteria of Landis and Koch[5]: less than 0, no agreement; 0.0 to 0.20, slight agreement, 0.21 to 0.40, fair agreement; 0.41 to 0.60, moderate agreement; 0.61 to 0.80, substantial agreement; 0.81 to 1.00, almost perfect agreement.

The diagnostic performance of the microfluidic system was evaluated by sensitivity, specificity, and positive and negative predictive values against the reference standard of cancer identified within surgically obtained formalin-fixed paraffin embedded biopsy or resection specimens of lung. Exploratory analyses were undertaken to help determine the optimum clinical classification: a sensitivity-weighted analysis (either pathologist classifies the results as suspicious or positive for cancer) and a specificity-weighted analysis (both pathologists classify the results as suspicious or positive for cancer).

Categorical data are presented as frequencies (%) and continuous data are presented as means with standard deviation (SD). Statistical analyses were performed using Stata version 10.0 (StataCorp, College Station, Tex).

RESULTS

From March 2011 to October 2012, 46 patients at our institution donated blood for research (Table 1). The mean age was 66 years (SD 12 years) and 22 (48%) were men. Cancer was present as an underlying diagnosis in 43 (94%); 34 (79%) had primary lung cancer and metastatic cancer of nonlung origin was diagnosed in 8 (19%). Of the 43 patients with cancer, 18 (42%) had stage I to II tumors and 22 (51%) had stage III to IV tumors. In 3 cases, the stage of the tumor was not identified. The size of 60% of tumors was category T1 or T2; 46% were lymph node positive and 49% were lymph node negative.

The proportion of patients with cancer in which atypical cells suspicious for cancer were identified on H&E staining was 16/43 (37%) by the principal pathologist and 10/17 (59%) by the second pathologist. Each pathologist also identified the cells in 1 of the 3 patients with benign disease they tested. The interobserver agreement was 80% corresponding to a kappa of 0.615 indicating substantial agreement.

Staging was reported clinically in 40 of 43 patients with cancer as a diagnosis (Table 1). Among these 40 patients, atypical cells suspicious for cancer on conventional light microscopy were identified in 6/18 (33%) patients with early stage (I/II) and 10/22 (45%) patients with advanced stage (III/IV) disease.

Using a sensitivity-weighted analysis, the test performance for the diagnosis of cancer yielded a sensitivity of 54% (95% confidence interval [CI], 37-72), specificity of 33% (95% CI, 2-91), a negative predictive value of 4% (95% CI, 1-19), and a positive predictive value of 93% (95% CI, 89-97). Using a specificity-weighted analysis, the test performance for the diagnosis of cancer yielded a sensitivity of 43% (95% CI, 21-71), specificity of 100% (95% CI, 5-100), a negative predictive value of 7% (95% CI, 2-29), and a positive predictive value of 95% (95% CI, 85-98).

DISCUSSION

Microfluidics is considered to be one of the most promising technologies to capture CTCs.[6] The advantage of microfluidic methods is that they can simultaneously take into account the physical and biological properties of CTCs, such as size and expression of tumor-specific biomarkers. Different microfluidic approaches are being developed and tested experimentally. This study assessed the size-filtration technique using a ClearCell CTChip (Figure 1), which consists of a chamber with 900 crescent-shaped pillar traps that can catch enlarged and less-deformable (stiffer) tumor cells, while allowing red blood cells and leukocytes to go through.[3] The biological features of the entrapped cells can be analyzed subsequently using appropriate staining (including immunocytochemistry); the cells can also be retrieved from the chip and used for downstream applications such as gene expression analysis, mutation detection, and cell culture.

TABLE 1. Baseline characteristics of the patients

Characteristic	Value
Sample size, n	46
Mean age, y (SD)	66 (12)
Men, n (%)	22 (48)
Cancer as an underlying diagnosis, n (%)	43 (94)
Primary lung cancer, n (%)	34 (79)
Metastatic lung cancer, n (%)	8 (19)
Other primary thoracic malignancy, n (%)	1 (2)
Benign lung disease, n (%)	3 (6)
Tumor grade	
Stage I to II, n (%)	18 (42)
Stage III to IV, n (%)	22 (51)
Insufficient information to classify stage, n (%)	3 (7)
Tumor size	
T1, n (%)	13 (30)
T2, n (%)	13 (30)
T3, n (%)	2 (5)
T4, n (%)	5 (12)
Unknown, n (%)	10 (23)
Lymph node status	
Positive, n (%)	20 (46)
Negative, n (%)	21 (49)
Unknown, n (%)	2 (5)
Smoking history	
Current smoker, n (%)	10 (22)
Ex-smoker, n (%)	16 (35)
Never smoker, n (%)	6 (13)
Unknown, n (%)	14 (30)

SD, Standard deviation.

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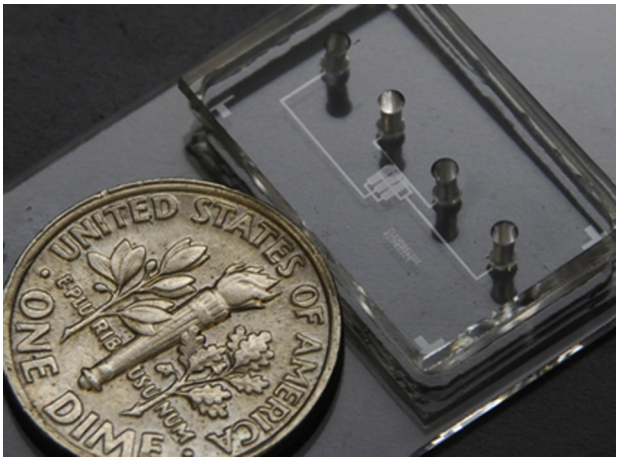

FIGURE 1. Microfluidic biopchip next to a US 10 cent coin.

Our study is the first clinical report in which microfluidic technology was used to capture atypical cells from peripheral blood by staining them using routine histologic stains and examining them using light microscopy. Although the clinical test performance was not perfect for sensitivity, excellent specificity was achieved using 1 mL of blood for diagnosing lung cancer. The samples analyzed were collected in a nonbiased prospective way without preliminary selection of patients by any criteria. Therefore, we believe that the sample reflects a common situation for clinicians in daily practice. As a drawback, the sample contains only 3 patients with benign lung disease, hence the wide confidence limits with the estimates of specificity.

Currently, a panel of antibody stains (such as EpCAM+, CD45−, DAPI+) are commonly applied to diagnose lung cancer using CTCs. However, this is not the accepted clinical definition as published by the World Health Organization[7] and epithelial (nonmalignant) cells also carry the same immunophenotype.[8] In an effort to develop a point-of-care blood-based diagnostic test, our focus is on replicating conditions that would facilitate conventional clinical diagnosis from blood; microfluidic technology seems to provide a (small) advance on current technology that cannot differentiate epithelial and cancer cell capture.

With regard to potential clinical application, the current test performance (relatively low sensitivity) suggests that as a negative result cannot rule out disease, the high specificity implies that patients who test positive most likely do have the disease. The test sensitivity is influenced by the number of patients (with suspected lung cancer) with CTCs in the blood, a parameter this is not well studied (and not under our control), and may be affected by the small amount of blood (1 mL) used. In addition, analysis and reporting of cells within a microfluidic chamber is a completely new method of reporting on cells that have been subject to shear stresses. Based on our data, we estimate that 33% of patients with early cancer and 45% of patients with advanced cancer have atypical cells identifiable in their peripheral blood. If this is the case, successful clinical implementation could define this as the proportion of patients who would not need to undergo confirmatory tissue biopsy. Currently, our estimates have a wide confidence interval because it was a proof-of-concept study.

At present, this technology is limited by an inability to definitively confirm that the atypical cells are from the primary tumor, but given their morphology, it seems likely that this is the case as the cells are larger than circulating lymphocytes and other white blood cells. The shear stress rendered from the microfluidic cell capture distorts the architecture of the cells and having to undertake light microscopy though a biochip is an additional a limitation. This may be one of the main reasons for disagreement between the pathologists in identification of the CTCs (20% in our study). Further refinements are underway to ensure better cell preservation and complete cell extraction with the application of immunocytochemistry (where required) to further characterize the captured cells in our efforts to achieve a clinical grade diagnostic test.

CONCLUSIONS

Our results demonstrate the potential of microfluidic technology to develop a highly specific blood test for the diagnosis of cancer in peripheral blood; conventional clinical criteria can be used as proof-of-concept of what can be achieved with today's technology.

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